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Formulation and Characterization of Silybum marianum phytosome for Treatment of Liver Cirrhosis

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ABSTRACT

Liver cirrhosis is a progressive and irreversible liver disorder characterized by fibrosis, regenerative nodules, and deterioration of hepatic function, leading to significant morbidity and mortality worldwide. Although Silybum marianum (Milk Thistle) is widely recognized for its hepatoprotective properties due to the presence of silymarin, its therapeutic efficacy is limited by poor aqueous solubility and low oral bioavailability. The present study aimed to formulate and characterize a Silybum marianum phytosome to enhance the bioavailability and therapeutic potential of silymarin for the management of liver cirrhosis. Ethanolic extraction of Silybum marianum was performed using the Soxhlet extraction method, followed by qualitative and quantitative phytochemical analyses to identify major bioactive constituents. The phytosome complex was prepared using phospholipids at optimized drug-to-lipid ratios and characterized through differential scanning calorimetry (DSC), solubility studies, UV-visible spectroscopy, FTIR analysis, vesicle size determination, zeta potential measurement, and in vitro drug permeation studies. Antioxidant activity was evaluated using the DPPH free radical scavenging assay. The extract demonstrated a high yield with abundant flavonoids, alkaloids, tannins, phenolics, and saponins. The phytosomal formulation exhibited enhanced solubility, improved membrane permeability, and superior antioxidant activity compared with the conventional extract, indicating improved bioavailability and stability. The findings suggest that phytosome technology effectively enhances the therapeutic performance of Silybum marianum, making it a promising herbal nanocarrier system for liver cirrhosis management. This formulation may serve as a safer and more effective alternative to conventional herbal preparations and warrants further preclinical and clinical investigations to establish its long-term efficacy and therapeutic applicability.

Keywords: Silybum marianum, Phytosome, Liver Cirrhosis, Silymarin, Hepatoprotective Drug Delivery

INTRODUCTION:

Liver cirrhosis is a progressive and chronic disease characterized by the replacement of healthy liver tissue with scar tissue (fibrosis), which leads to impaired liver function. It represents the end stage of various chronic liver diseases and is a significant global health concern. According to the World Health Organization (WHO), cirrhosis accounts for overone million deaths annually, ranking as one of the top causes of mortality worldwide (WHO, 2020).

Cirrhosis develops as a result of sustained liver injury from a variety of causes, including chronic viral hepatitis (hepatitis B and C), alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis, and metabolic disorders such as Wilson's disease and hemochromatosis. These conditions lead to inflammation, necrosis, and fibrosis of liver cells, which disrupts the normal architecture of the liver and impairs its regenerative capacity (Schuppan & Afdhal, 2008).

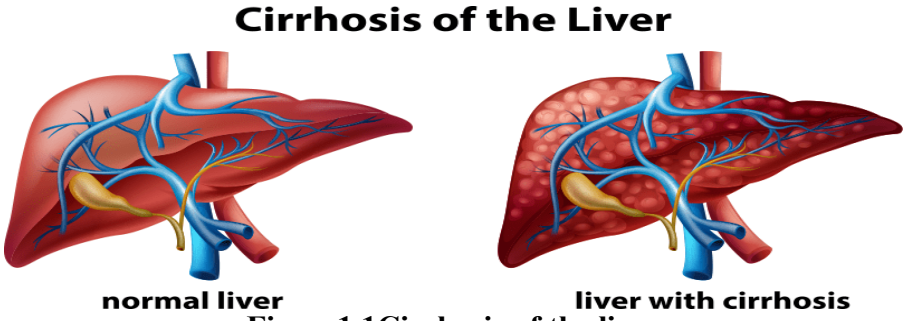

Figure1.1Cirrhosis of the liver

The pathogenesis of cirrhosis is complex and involves dynamic interactions between hepatocytes, Kupffer cells, stellate cells, and immunemediators. Activation of hepatic stellate cells, which play a central role in fibrosis, is a hallmark of cirrhotic progression (Tacke & Weiskirchen, 2012). As scar tissue accumulates, portal hypertension, liver dysfunction, and complications such as variceal bleeding, ascites, hepatic encephalopathy, and hepatocellular carcinoma (HCC) may ensue (Gines et al., 2004).

In addition to its significant morbidity and mortality, cirrhosis imposes a substantialeconomic burden due to hospitalization costs, complications, and the need for liver transplantation in advanced cases (D'Amico et al., 2006). Early detection, management of complications, and treatment of underlying causes are critical in slowing disease progression and improving patient outcomes.

2.PATHOPHYSIOLOGY OF LIVER CIRRHOSIS

Liver cirrhosis is a chronic, progressive, and irreversible pathological condition characterized by diffuse fibrosis, formation of regenerative nodules, and distortion of the normal hepatic architecture.

It represents the final common pathway of various chronic liver diseases resulting from persistent liver injury.

Figure1.2 Pathophysiology of Liver Cirrhosis

Normal Liver Structure and Function

The normal liver is composed of hepatocytes arranged in lobules around a central vein. Blood from the portal vein and hepatic artery flows through hepatic sinusoids, allowing hepatocytes to perform vital metabolic, synthetic, detoxification, and excretory functions. Hepatic stellate cells remain in a quiescent state under physiological conditions and store vitamin A.

Initiationof Liver Injury

Cirrhosis begins with **repeated or continuous liver injury**,commonly due to:

- Chronic alcohol consumption
- Viral hepatitis(HBV,HCV)
- Non-alcoholic fatty liverdisease(NAFLD/NASH)
- Autoimmune liver diseases
- Chronicbiliary obstruction
- Drug-toxin-induced liver damage

Persistent injury leads to hepatocyte necrosis and apoptosis, triggering inflammatory responses within the liver (Friedman SL. 2003).

Inflammatory Response

Damaged hepatocytes release damage-associated molecular patterns (DAMPs), which activate

Kupffer cells (resident hepatic macrophages). Activated Kupffer cells secrete pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukins (IL-1, IL-6), and transforming growth factor-β (TGF-β).

These mediators promote:

- Recruitment of inflammatory cells
- Sustained hepatic in flammation
- Activation of fibrogenic pathways(SchuppanDetal., 2008)

Activation of Hepatic Stellate Cells

A central event in cirrhosis development is the **activation of hepatic stellate cells (HSCs)**. Under chronic inflammatory stimulation, stellate cells transformintomy fibroblast-like cells.

Activated HSCs:

- Proliferaterapidly
- Lose vitamin A storage
- Produce excessive extracellular matrix(ECM)component ssuch as collagen typesI and III

This excessive collagen deposition disrupts normal sinusoidal blood flow.

Progressive Fibrosis Formation

Continuous ECM accumulation exceeds its degradation resulting in:

- Bridging fibrosis between portal tracts and central veins
- Thick fibrous septa formation
- Compression of hepatic sinusoids

Over time, fibrosis replaces functional liver parenchyma, significantly impairing hepatic function (Bataller R et al., 2005).

Regenerative Nodule Formation

As hepatocytes attempt to regenerate in response to injury, they proliferate within fibrotic bands, forming **regenerative nodules**. However, these nodules lack normal vascular connections, leading to ineffective regeneration.

The combination of fibrosis and nodular regeneration produces **architectural distortion**, which is the hallmark of cirrhosis.

Functional Consequences of Cirrhosis

Structural distortion results in severe functional impairment:

↓Protein synthesis→hypo albuminemia, edema

↓Clotting factor synthesis→bleeding tendency

↓Detoxification →accumulation of ammonia leading to hepaticencephalopathy

↓Bileexcretion→jaundice and pruritus

Symptoms

- Extreme tiredness.
- Easily bleeding or bruising.
- Loss of appetite.
- Nausea.
- Swelling in the legs, feet or ankles, called edema.
- Weight loss.
- Itchy skin.
- Yellowing of the skin and eyes, called jaundice.
- Fluid build up in the belly, called as cites(uh-SY-teez).

3.PLANT PROILE

Silymarin is a well-known herbal hepato protective agent obtained from these sand fruits of *Silybum marianum* (L.) Gaertn., commonly known as Milk thistle. It has been traditionally used for the treatment of liver disorders such as hepatitis, cirrhosis, fatty liver disease, and toxin-induced hepatic damage. Silymarin is widely employed in modern medicine due to its antioxidant, anti-inflammatory, antifibrotic, and hepato regenerative properties (Kren V et al., 2005).

Botanical Source

Biological source: Silymarin is obtained from thedried seeds (achenes) of *Silybummarianum* (L.) Gaertn.

Family:Asteraceae (Compositae)(FloraKetal.,1998)

TaxonomicalClassification

Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Silybum
Species	Silybummarianum

Figure1.4: Silymarin (MilkThistle)

Synonyms: Milkthistle , Holythistle , Marianthistle ,Carduusmarianus

Geographical Distribution

Silybum marianum is native to the Mediterranean region and Southern Europe. It is widely cultivated in: India, Germany, Austria, Hungary, China, United States.

Morphological Characteristics Plant type:

Biennial herb Height: 1–2 meters

Leaves:Large,glossy green leaves with white marbling and spiny margins

Flowers: Purple or reddish flower heads surrounded by spiny bracts Fruits: Brown to black achenes with a white pappus

Chemical Constituents

Silymarin is a mixture of flavonolignans,mainly consisting of:

Silybin(Silibinin)–majoractive constituent

- Isosilybin
- Silychristin
- Silydianin
- Taxifolin(flavonoid)

Among these,silybina ccounts for nearly50–70%of total silymarin and exhibits the strongest hepatoprotective activity.

Mechanism of Action

Silymarinexertshepatoprotectiveactivitythroughmultiplemechanisms:

- Acts as a powerful antioxidant by scavenging free radicals
- Inhibits lipid peroxide at ionin hepatocyte membranes
- Enhances protein synthesis by stimulating RNA polymeraseI
- Stabilizes hepatocyt cell membranes
- Inhibits hepatics tell at cell activation,there by reducing fibrosis
- Promotes regeneration of damaged livercells (PradhanSC etal.,2006)

PharmacologicalActivities

- Hepatoprotective
- Antioxidant
- Anti-inflammatory
- Antifibrotic
- Antiviral(against hepatitis viruses)
- Anticancerpotential
- Immunomodulatory activity

TherapeuticUses

- Liver cirrhosis
- Alcoholic liver disease
- Non-alcoholic fattyliver disease (NAFLD)
- Drug-andtoxin-induced hepatotoxicity
- Viral hepatitis
- Mushroom poisoning (*Amanitaphalloides*)(BrunetonJ.1999)

PHYTOSOMES

Herbal medicines have been extensively used in traditional systems of medicine for centuries due to their wide range of therapeutic activities and relatively better safety profiles. Plant-derived phytoconstituents such as flavonoids, alkaloids, terpenoids, glycosides, tannins, and polyphenols have demonstrated significant pharmacological properties including antioxidant, anti-inflammatory, hepatoprotective, anticancer, antimicrobial, and neuroprotective effects (Mukherjee PK et al., 2023 and Gupta M et al., 2024). Despite these promising biological activities, the clinical application of many herbal drugs remains limited due to poor bioavailability, instability, rapid metabolism, and low lipid solubility (Semalty A et al.,2010).

Most phytoconstituents are polar in nature and possess large molecular sizes, which restrict their permeability across biological membranes, particularly the gastrointestinal epithelium. As a result, conventional herbal extracts often show poor absorption and inconsistent therapeutic outcomes when administered orally (Jain N et al., 2010). These limitations have created a need for advanced drug delivery systems capable of enhancing the bioavailability and therapeutic effectiveness of herbal medicines.

The term *phytosome* is derived from the Greek words *"phyto"* meaning plant and *"soma"* meaning body or cell-like structure. Phytosomes are defined as lipid-compatible molecular complexes formed by the interaction between phytoconstituents and phospholipids, primarily phosphatidylcholine (Kalita B et al., 2019). In this system, the active plant constituent forms hydrogen bonds with the polar head of the phospholipid, resulting in a stable complex with improved lipophilicity.

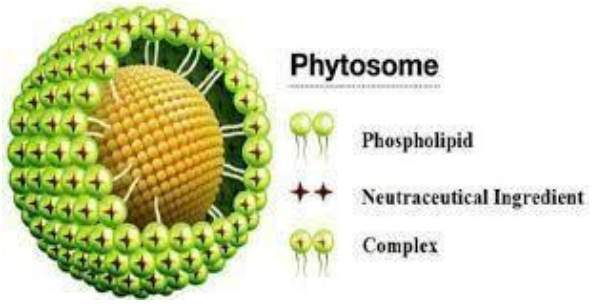

Figure1.5: Pytosome

Unlike liposomes, where the drug is physically entrapped within the vesicle, phytosomes involve a stoichiometric complex formation between the phytochemical and phospholipid, usually in a 1:1 or 1:2 molar ratio (Kumar A et al. 2017). This structural arrangement significantly enhances the ability of phytoconstituents to cross biological membranes, thereby improving systemic absorption.

Advantages of Phytosome Technology

Phytosome-based drug delivery systems offer several advantages over conventional herbal formulations, including:

- Enhanced oral bioavailability of poorly absorbed phytoconstituents
- Improved stability against enzymatic and chemical degradation
- Better membrane permeability due to lipid compatibility
- Reduced dose requirement and improved therapeutic efficacy

Applicability in oral, topical, and transdermal drug delivery systems (Sharma Setal., 2023 and Patel J et al., 2024)

Therapeutic Applications of Phytosomes:

Phytosome technology has been successfully applied to various herbal drugs such as silymarin, curcumin, quercetin, rutin, green tea polyphenols, ginkgo biloba, and ginseng. These phytosomal formulations have demonstrated improved pharmacokinetic profiles, enhanced therapeutic activity, and better patient compliance (Pawar HA et al., 2025).

Recent research has expanded the application of phytosomes in the management of chronic diseases including liver disorders, inflammatory conditions, metabolic syndrome, cancer, and neurodegenerative diseases (Panda VS et al., 2020). Additionally, phytosomes have found increasing application in cosmeceuticals for enhanced dermal penetration and skin rejuvenation therapies (Djekic L et al.,2023).

MATERIALS

Selection of plants

One potential herbal plant selected for study is *Silybum marianum*.

Collection of plants

The whole plants of *Silybum marianum* will be collected in a fresh condition from the local area of Chhattisgarh. The whole plant of *Silybum marianum* was washed with pure water and was completely dried in the shade for 10 days. Then the plant was crushed into porous powder and stored.

Reagents and Solvents

All the reagents and solvents were purchased from Molychem, Loba Chemie All Chemicals, reagents, and solvents used for experimentation, along with their suppliers, are given below (Table 5.1).

Table5.1 List of reagents and solvents with their CAS Number and suppliers

S. No.	Reagentsand Solvents	Supplier	CAS Number
1	Ethanol	Chemical Support Systems (CSS)	64-17-5
2	Methanol	Loba Chemie	67-56-1
3	Chloroform	Oualigens	67-66-3
4	EthylAcetate	Molychem	141-78-6
5	n-Hexane	Molychem	110-54-3
6	Dragendorff's Reagent	Molychem	39775-75-2
7	Folin-ciocalteu Reagent	CDH (Central Drug House)	12111-13-6
8	Acetone	Molychem	67-64-1
9	Diethyl ether	LobaChemie	60-29-7
10	Benedict's Reagent	Molychem	63126-89-6

METHODS

1. Extraction

The plant extract was prepared using the Soxhlet method as described. A 152g powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as the solvent. The temperature was maintained at 75 °C throughout the 12-hour extraction period. The crude extract was concentrated in a vacuum using a rotary evaporator. The concentrated extract was subsequently dried aseptically at room temperature.

The percentage of yield of the extract was calculated as:

$$\%\text{Yield } (\%Y) = \frac{\text{Weight of dried extract (g)}}{\text{Weight of sample (g)}} \times 100$$

Figure5.1:Soxhletion

2. Solubility Profile

To find out how the extract of *Silybum marianum* performed in different solvents, solubility testing was performed. The formulation design, biological activity testing, and phytochemical analysis are all facilitated by this study's assistance in selecting suitable solvents. Using visual observation of extract dispersion and clarity in several solvent systems, solubility was qualitatively determined.

To test for solubility, 5 mL of each chosen solvent was mixed with approximately 100 mg of dried ethanolic extract in different test tubes. At room temperature, the mixtures were shaken well, and their solubility was checked ten minutes later.

Solvents Used:

Distilled Water, Ethanol, Acetone, Chloroform, n-Hexane, Ethyl acetate, Diethyl ether

3.PhytochemicalScreening

The preliminary screening will help detect major phytochemical groups such as alkaloids, flavonoids, terpenoids, phenolics, tannins, and saponins. The extract obtained from Soxhlet extraction using ethanol will be subjected to the following standard phytochemical tests (Dev Sharma *et al*, 2023). If

positive results are obtained, it will confirm the presence of these bioactive compounds in *Silybum marianum.*

Qualitative Analysis

The concentrated extracts of the selected plant were subjected to different chemical tests for thedetection of different phytoconstituents using standardmethods.(Karki et al., 2020; Lee et al., 2013; Sharma et al., 2023).

Test for Alkaloids

1.Dragendorff's Test: In 1 ml of extract solution, a few drops of Dragendorff's reagent were added,and the colour developed was observed. The appearance of orange colour indicates the presence of Alkaloids.

Testfor Flavonoids

Alkaline Test: In test tubes, 2 mL of extract was added, along with 2 mL of 10 % NaOH.The development of bright yellow colour and the disappearance of yellow colour when adding diluted HCl suggested the presence of flavonoids.

Test for Tannin & Phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of a bluish black colour indicates the presence of phenol.

Test for Saponins

FoamTest: 0.5 g of extract was shaken with 2 ml of water. If foam persists for ten minutes,it indicates the presence of saponins.

Test for Terpenoids

Salkowski's Test: After taking 3 ml of the extract, 1ml of chloroform and 1.5 ml of concentrated H2SO4 were added along the tube edges. The reddish-brown colour indicates the presence of terpenoids.

Test for Glycosides

Keller-Killiani Test: 1 ml of the extract was combined with 1 ml of glacial acetic acid, 2/3 drops of 5%FeCl3,and 1ml of concentrated H2SO4.The interface displays a brown,green, or violet ring indicating the presence of glycosides.

Test for Carbohydrates

Benedict's Test:

Filtrates were treated with Benedict's reagent and heated gently. Orange-red colored precipitate indicates the presence of reducing sugars.

Test for Proteins & Amino Acids

Biuret Test: To the 2mL filtrate, 1 drop of 2% copper sulphate solution and 1mL of 95% ethanol, along with NaOH pellets, were added, and Formation of violet colored solution formed in the ethanolic layer.

Ninhydrin Test: 2ml of ninhydrinre agent to 2 ml of extract, and boil for a few minutes. The presence of amino acids is indicated by the development of purple or blue colour.

Test for Fats &Oils

Spot Test: A small quantity of extracts was pressed between two filter papers. Formation of a grease spot indicates the presence of fixed oils and fats.

Quantitative

Phytochemical analysis of the ethanolic extracts was unde rtaken using standard methods as described by Edeoga, Trease and Evans, Harborne, Daniel and Prasthith.

Determination of Total Flavonoids

Quercetin was used as the standard in the method of calculating total flavonoid content, with some modifications. The quercetin calibration curve was created in the range of 0–200mg/ml. In short, two test tubes were filled with extract (0.5 mL) and standard (0.5 mL). To each test tube, 10% aluminium chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL), and distilled water (2.8 mL) were added and combined. 0.5 mL of distilled water was used in place of the sample or standard to create a blank, and the same procedure was followed to substitute distilled water for the aluminium chloride. For thirty minutes, each tube was incubated at room temperature. At 415nm, the absorbance was measured with a UV-visible spectrophotometer. Each gram of the extract's flavonoid content was measured in milligrams of quercetin equivalent (QE) (Meenatchi et al., 2017).

$$\mathbf{Y = Mx + C}$$

where,

Y – Absorbance, **M** - Slope of the calibration curve, **X** - Concentration of tannins in the test solution, **C** - Intercept of the calibration curve.

Determination of Total Phenolics

Folin-Ciocalteu's technique was used with a spectrophotometer to measure the total phenolic content. Regarding plant extract, one millilitre of Folin-Ciocalteu's phenol reagent was combined with one millilitre of the sample (1 mg/ml). Following five minutes, 13 ml of deionised distilled water and 10 millilitres of a 7% Na2CO3 solution were added and thoroughly mixed. For the reaction (blue colour form), the mixture was left in the dark at 23°C for 1.30 hours. A dilution of 10, 20, 30, 40, 50, and 60 g/ml of gallic acid wasmade asa reference, and the absorbance was measured at 765 nm (Chandran& Kavitha Chandran, 2016). A calibration curve extrapolation was used to calculate the total phenolic content, which was then expressed as milligrams of gallic acid equivalent per gram of extract. The following formula was used to estimate the phenolic compounds.

$$\mathbf{Y = Mx + C}$$

where,

Y–Absorbance, **M** –Slope of the calibration curve, **X**-Concentration of tannins in the test solution, **C** - Intercept of the calibration curve.

Determination of Total Alkaloid

The method outlined by (Winitchaikul et al., 2021) was modified to analyse the total alkaloid content. One millilitre of 2 N HCl was used to dissolve the sample (10 mg), which was then extracted three times (3, 3, and 4 mL) into chloroform. Thelayers of chloroform were thrown away. After neutralising the HCl layers with 0.1 N NaOH, 5 mL of bromocresol was added. Green solution with 5 millilitres of pH 4.7 phosphate buffer solution. Chloroform was used three times to extract this mixture (3, 3, and 4 mL). After combining the chloroform layers, the absorbance was determined to be 420 nm. By comparing the absorbance with a berberine chloride (SigmaAldrich,USA)standard curve, the total alkaloid content was determined within the range of 2−16 μg/mL (Y =0.0342X −0.0223, R 2 = 0.9946), where Y stands for the berberine chloride absorbance at 420 nm, X for the berberine chloride concentration (μg/mL), and R for the linear correlation coefficient. Mg BCE/10 g extract, or milligrammes of berberine chloride equivalents per 10 g extract, was used to express the overall alkaloid concentration.

$$\mathbf{Y = Mx + C}$$

where,

Y–Absorbance, **M** –Slope of the calibration curve, **X**-Concentration of tannins in the test solution, **C** - Intercept of the calibration curve

Determination of Total Tannin

The Folin-Ciocalteu method was used to determine the tannins. A volumetric flask (10 ml) was filled with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution, and 0.1 ml of the sample extract. The flask was then diluted to 10 ml with distilled water. After giving the mixture a good shake, it was allowed to sit at room temperature for half an hour (Thakre et al., 2024). Tannic acid reference standard solutions (20, 40, 60, 80, and 100 µg/ml) were made using the previously mentioned technique. An ultraviolet/visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 700 nm, about the blank. Threse separate measurements were made in order to estimate the tannin content. The amount of tannic acid equivalents per gram of dried sample was used to express the tannin concentration (Indriaty et al., 2023).

$$\mathbf{Y = Mx + C}$$

where,

Y – Absorbance, **M** - Slope of the calibration curve, **X** - Concentration of tannins in the test solution, **C** - Intercept of the calibration curve

Determination of Total Saponin Content

In a conical flask, 20 g of the extract was combined with 100 mL of 20% aqueous ethanol. The mixture was heated to 55 °C for four hours while being constantly stirred in a water bath. 200millilitres of 20% ethanol were used to filter and re-extract themixture.Usingawater bath set to 90 °C, the filtrates were mixed, and the volume was lowered to 40 mL. 20 millilitres of diethyl ether were added to a 250 ml separating funnel containing the concentrated filtrate. After giving this a good shake, it was permitted to split into two layers. The aqueous layer was recovered after this extraction was completed three times. Using 60 mL of n-butanol, the aqueous layer was extracted three times. Ten millilitres of 5% NaClwere used to wash the n-butanol extract three times. In order to evaporate the n-butanol, the cleaned n-butanol extract was heated in a water bath. To produce the saponins, the n-butanol extract was dried in an oven set at 50 °C to a consistent weight. After mixing 50 µL of the n-butanol extract with 0.2 mL of vanillin-acetic acid (5% w/v) and 0.8 mL of perchloric acid, the mixture was baked for 15 minutes at 70 °C.Five millilitres of glacial acetic acid were added to the mixture after it had been chilled for one minute in an ice bath. After that, the combination was examined at a wavelength of 550 nm using a UV/Vis spectrophotometer (Edewor et al., 2016).

$$Y = Mx + C$$

where,

Y – Absorbance, **M** - Slope of the calibration curve, **X** - Concentration of tannins in the test solution, **C** - Intercept of the calibration curve.

Determination of Total Ash Value

To determine the total Ash value, 1 g of precisely weighed dry plant powder was used, and it was then incinerated in a porcelain crucible at a temperature of no more than 450°C until it turn edentirely white. After allowing the crucible to cool, it was weighed. A constant weight was achieved by continuing this method. The dried material's total ash content was determined to be mg/g, and the percentage was then displayed (Das et al., 2022; Nayeem et al., 2020; Sadiq, n.d.).

$$\%\text{Total Ash Value} = \frac{\text{Ash weight}}{\text{Weight of Sample}} \times 100$$

Preparation and Characterization of Phytosomes:

Material

The phospholipids, hydrogenated soy Phosphatidyl choline (HSPC) was purchased from Lipoid, Ludwigshafen, Germany. Gallic acid and 1, 1-Diphenyl-2-picrylhydrazyl (DPPH)was purchased from Sigma (Sigma Chemical, St. Louis, MO, USA); n- octanol and n-hexane were purchased from SRL chemicals, Mumbai, India. Another chemical was of analytical grade.

Preparation of Phytosomes

The complex was prepared with phospholipids and both the drugs gallic acid and rutin as a molar ratio of 1:1, 1.5:1, 2:1, 2.5:1and 3:1 respectively. Weight amount of drug and phospholipids were placed in a 100ml round-bottom flask and 50ml of methanol was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces number of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle127.Process variables used for optimization The developed formulation was optimized by selecting following process variables.

- Effect of ethanol concentration
- Effect of lecithin concentration
- Drug concentration

Characterization

Differential Scanning Calorimetry (DSC)

The samples were sealed in the aluminum crimp cell and heated at the speed of 10◦C/min from 0 to 300◦C in nitrogen atmosphere (60 ml/min). The peak transition onset temperature of gallic acid, phospholipid, gallic acid–phospholipid complex and physical mixture of gallic acid and phospholipid were determined and compared with the help of a Mettler DSC 30 S (Mettler Toledo, UK).

Solubility Studies

Determination of solubility characteristics of gallic acid, gallic acid–phospholipid complex and physical mixture of gallic acid and phospholipid were obtained by adding excess of the samples to 5ml of water or *n*-octanol in sealed glass container at room temperature. The liquids were shaken for 24 h and centrifuged at 5000 rpm for 10 min. The supernatant was filtered, and 1ml of filtrate mixed with 9 ml of methanol. Twenty micro-liters aliquot of the resulting solution injected in HPLC and concentration of gallic acid was measured at 360 nm.

Determination of Interaction between Gallic Acid and Phospholipids

UV analysis was performed on a UV-visible spectrophotometer (Shimadzu-UV 1700 Japan) and Fourier transform infrared spectrophotometer (FT-IR Spectrometer, Bruker Ifs-55, Switzerland) was used to study the interaction between gallic acid and phospholipids. The IR spectra of gallic acid, phospholipids, their complex and physical mixture were obtained by the KBr method.

Vesicle size and size distribution

The vesicle size, size distribution and zeta potential of optimized Phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zeta master ZEM 5002, Malvern, UK). The electric potential of the Phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

***Invitro* drug permeation study**

Invitro permeation study of the all formulations and market preparation was carried out using modified Franz diffusion cell. This experiment is generally carried out by using skin membrane however we had replaced the skin membrane by a prepared biological membrane.

Specification of franz diffusion cell

Modified Franz diffusion cell used was made from borosilicate glass. Specification of cell is as given in Table 4.1.

Preparation of biological membrane

In this experiment we have used egg membrane as the required biological membrane. Egg membrane was prepared by method reported by Ansari et al (2006)130, at first egg was taken and then a small hole was made on the egg shell to expel the content. The entire egg was immersed in conc. HCl solution for an hour to dissolve the hard calcified layer without any further process. After the decalcification of the outer shell of the egg, delicate inner membrane containing the inner content was taken out cautiously.

4.6.1Free radical scavenging activity by DPPH Method

Free radical scavenging potentials of the gallic acid Phytosomes were tested against a methanolic solution of α, α-diphenyl-β-picryl hydrazyl (DPPH). Antioxidants reacts with DPPH and convert it to α,α-diphenyl-β-picrylhydrazine.The degree of discoloration washed using normal saline solution (0.9% NaCl). This prepared membrane was stored in refrigerator while dipping in normal saline solution for further used during experimental work.

Procedure for *invitro* drug permeation study

The *in vitro* permeation study is done by using modified Franz diffusion cell with previously treated egg membrane. Then this study was performed with phosphate buffer saline (pH 7.4). Modified Franz diffusion cell is a glass apparatus consist of two separate parts, the upper part is known as donor compartment and the lower part is known as receptor compartment. The receptor part contains a long side tube known as sampling tube through which samples are taken out for estimation. The receptor media was placed inside the receptor compartment and a magnetic bead was also placed inside. In between receptor and donor compartments, prepared biological membrane was placed and the joint of the two compartments was clamped properly. The formulation was placed (equivalent to 2.5 mg of drug) on the upper side of egg membrane in donor compartment. The whole assembly was attached on a magnetic stirrer cum heater unit and switched on. The temperature of the assembly was maintained at 37±2°C. Samples were withdrawn after every hour by taking 1 ml of the receptor media through the sampling tube and at the same time, same amount of fresh receptor media was added to maintain the sink condition. Samples were diluted up to 5 ml using fresh receptor media and its absorbance was measured by UV/visible spectrophotometer (Shimadzu-UV 1700 Japan) at 273.5 nm.

***In vitro* free radical scavenging and antioxidant screening**

Materials: α, α-diphenyl-β-picryl hydrazyl (DPPH), egg phospatidyl choline, β-carotene, and γ-linoleic acid were obtained from Sigma Chemical Co. (St.Louis, MO, USA). BHT (butylated hydroxy toluene), ascorbic acid, Tween-40, deoxy-d-ribose, trichloroacetic acid (TCA), and thiobarbituric acid

(TBA) were obtained from Hi-Media Labs (Mumbai, India). 1,10-o-phenanthroline, ferric chloride (FeCl3), ammonium molybdate, and sodium dithionite were obtained from Ranbaxy Fine Chemicals (New Delhi, India).indicates the scavenging potentials of the antioxidant extract.The change in the absorbance produced at 517 nm has been used as a measure of antioxidant activity131.

Materials:

DPPH stock solution:39.4mg of DPPH was dissolved in one liter of analytical grade methanol.

Preparation of the test sample: 10mg of the sample was dissolved in10ml of the methanol to make a stock solution (1000 µg/ml) separately. Different concentrations10,20,30,40,50,60,70, 80 and 90µg/ml samples were prepared from stock solution (1000µg/ml) separately.

Aliquots sample was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 ml respectively from stock solution of 1000 µg/ml and diluted up to 10 ml with methanol in 10 ml volumetric flask separately.

Preparation of Standard solutions*:* BHT (Butylated Hydroxy Toluene) and ascorbic acid (ASC) were taken as standard. 25 mg dissolved in 20 ml methanol and volume made up tothe mark in 25 ml volumetric flask.

Procedure: In case of gallic acid Phytosomes and rutin Phytosomes aliquots of 25, 50,100, 150, 200,300, 400 and 500 µl of Phytosomes and 25, 50, 75, 100 and 150 µl of standard solutions were taken in different test tubes, volume was adjusted to 500 µl. To this 5 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 minutes. The control was prepared as above without extract. The readings were read at 517 nm using methanol as blank. The absorbance of control was first noted at 517nm. The change in absorbance of the samples was measured. 5 to 50 µl (5 to 50 µg) of ascorbic acid and 10 to 80 µl (10 to 80 µg) of BHT were taken in different test tubes. The above concentrations of phytosomes and standard were taken in different test tubes, volume was adjusted to 1000 µl. To this 4 ml of methanolic solution of DPPH was added, shaken well and the mixture was allowed to stand at room temperature for 20 minutes. The control was prepared as above without phytosomes. The readings were read at 517 nm using methanol as blank. The absorbance of control was first noted at 517 nm. The change in absorbanceofthesamples was measured. Scavenging activity was expressed as the inhibition percentage calculated using the following formula,

$$\%\text{Anti radicalactivity} = \frac{\text{CONTROLAbs.} - \text{SAMPLE Abs.}}{\text{CONTROLAbs.}} \times 100$$

Each experiment was carried out in triplicate and percentages cavenging activities of various extracts are listed in the **Table and Graph.**

RESULTSANDDISCUSSION

Extractive Value

Phytochemicals are extracted from the whole plant sample using the Soxhlet process, and the resulting extract is stored at room temperature for later use. The extract yield following the concentration is found to be 38%.

Solubility Test

The solubility test shows that the extract is soluble in water at room temperature and becomes freely soluble at a hot temperature. In ethanol, the extract is soluble at room temperature and remains freely soluble when heated. In acetone, it is partially soluble at room temperature but becomes soluble at hot temperatures. The extract is freely soluble in diethyl ether, chloroform, and ethyl acetate at both room and hot temperatures. In n-hexane, the extract is soluble at room temperature and freely soluble at hot temperatures.

S. No.	Solvent	RoomTemp.	HotTemp.
1.	Water	++	+++
2.	Ethanol	++	+ ++
3.	Acetone	+	++
4.	Diethyl ether	+ ++	+ ++
5.	Chloroform	+ ++	+ ++
6.	Ethyl acetate	+ ++	+ ++
7.	N-Hexane	++	+ ++

Soluble(++), Freely Soluble(+++),Partial Soluble(+), Insoluble (-)

Table6.1 Solubility Test

Qualitative Phytochemical Analysis

The analysis of the phytochemicals present in the ethanolic extracts of the *Silybum marianum* whole plant sample confirmed the presence of the various phytochemicals listed in Table 11.

Phytochemical	TestName	Observation
Alkaloids	Dragendorff'sTest	+
Flavonoids	Alkaline Reagent Test	+

Tannins&Phenolics	Ferric Chloride Test	+
Saponins	FrothTest	+
Terpenoids	SalkowskiTest	+
Glycosides	Keller-KillianiTest	+
Carbohydrates	Benedict'sTest	+
Proteins&Amino Acids	Biuret Test NinhydrinTest	- -
Fats&Oils	SpotTest	+

(+)Present and(-) Absent

Table6.2 The analysis of the phytochemicals

Quantitative Phytochemical Analysis

Silybum marianum ethanolic extract contained flavonoids, saponins, tannins, and phenol, according to preliminary phytochemical screening of succeeding extracts. The quantitative phytochemical analysis of *Silybum marianum* extract reveals that the total flavonoid contentis 90.61 ± 13.54 QE µg/ml, the total phenolic content is 44.55 ± 1.38 GAE µg/ml, the total alkaloid content is 70.86 ± 3.26 AE µg/ml, and the total tannin content is 52.01 ± 0.56 TAE µg/ml, indicating that flavonoids are present in the highest concentration, followed by alkaloids, tannins, and phenolics.

Estimation of Total Flavonoid Content

The standard absorbance values of quercetin are recorded at different concentrations ranging from 0.2 to 2 µg/ml. At 0.2 µg/ml, the absorbance is 0.045, which gradually increases with concentration. The absorbance values are 0.148 at 0.4 µg/ml, 0.259 at 0.6 µg/ml, 0.338 at 0.8 µg/ml, 0.457 at 1.0 µg/ml, 0.573 at 1.2 µg/ml, 0.659 at 1.4 µg/ml, 0.717 at 1.6 µg/ml, 0.821at 1.8 µg/ml, and 0.949 at 2.0 µg/ml, indicating a linear relationship between concentration and absorbance. The *Silybum marianum* extract sample shows absorbance values of 0.359, 0.367, and 0.478 in triplicate readings. From the calibration curve, the total flavonoid content of the extract is calculated as 90.61µg quercetine quivalent (QE)/ml.The mean value is 90.61 with a standard deviation of 13.54, and the final result is expressed as 90.61 ± 13.54QE µg/ml.

Table6.3 StandardAbsorbanceof Quercetin

S. No.	Standard Concentration (µg/ml)	Absorbance
1.	0.2	0.045
2.	0.4	0.148
3.	0.6	0.259

4.	0.8	0.338
5.	1	0.457
6.	1.2	0.573
7.	1.4	0.659
8.	1.6	0.717
9.	1.8	0.821
10.	2	0.949

Table 6.4 Total Flavonoid Content of *Silybum marianum* extract

Sample	Absorbance (y)	Mean	Standard Deviation	Mean±SD (QE µg/ml)
S.M. 1	0.359	90.61	13.54	90.61 ± 13.54
S.M. 2	0.367			
S.M. 3	0.478			

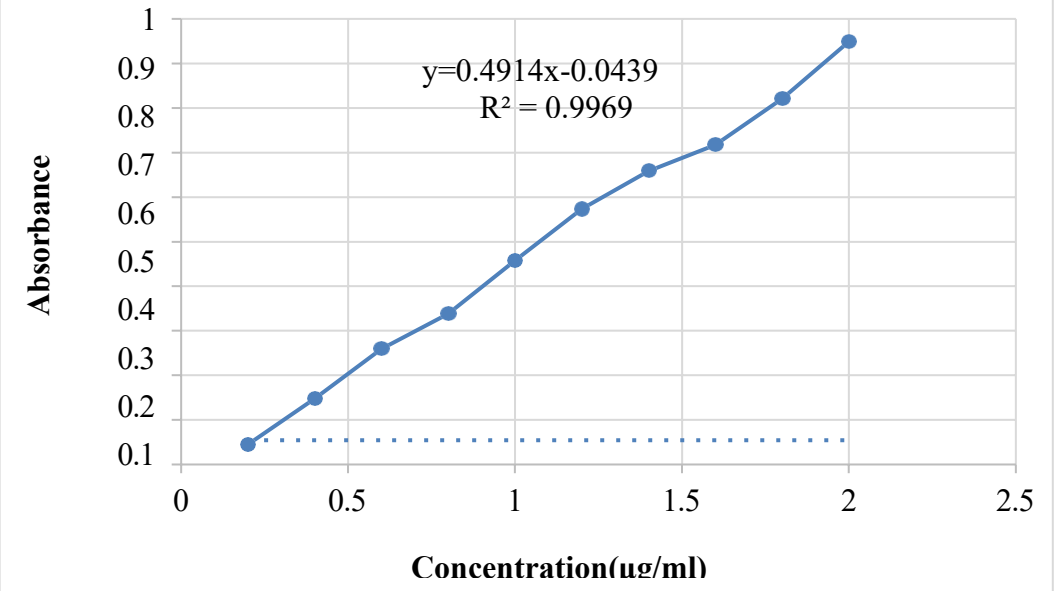

Fig6.1 Graph-Standard Curve of Quercetin

Estimation of Total Phenolic Content

The standard absorbance values of gallic acid are recorded at concentrations ranging from 0.2 to 2.0 µg/ml. At 0.2 µg/ml, the absorbance is 0.163, and it increases with concentration. The absorbance values are 0.232 at 0.4µg/ml,0.370 at 0.6µg/ml,0.412at 0.8µg/ml,0.512at1.0 µg/ml, 0.663 at 1.2µg/ml ,0.769 at 1.4µg/ml ,0.843 at 1.6µg/ml,0.963at1.8µg/ml,and 1.061 at 2.0 µg/ml, indicating a linear

increase in absorbance with concentration. The *Silybum marianum* extract shows absorbance values of 0.260, 0.267, and 0.274 in triplicate readings. From the calibration curve, the total phenolic content of the extract is calculated as 44.55 µg gallic acid equivalent (GAE)/ml. The mean value is 44.55 with a standard deviation of 1.38, and the final result is expressed as 44.55 ± 1.38 (GAE µg/ml).

S.No.	Standard Concentration (µg/ml)	Absorbance
1.	0.2	0.163
2.	0.4	0.232
3.	0.6	0.370
4.	0.8	0.412
5.	1.0	0.512
6.	1.2	0.663
7.	1.4	0.769
8.	1.6	0.843
9.	1.8	0.963
10.	2.0	1.061

Table6.5 Standard Absorbance of Gallic acid

Sample	Absorbance (y)	Mean	Standard Deviation	Mean±SD (GAEµg/ml)
S.M. 1	0.26	44.55	1.38	44.55 ± 1.38
S.M. 2	0.267			
S.M. 3	0.274			

Table6.6 Total Phenolic Content of *Silybum marianum* extract

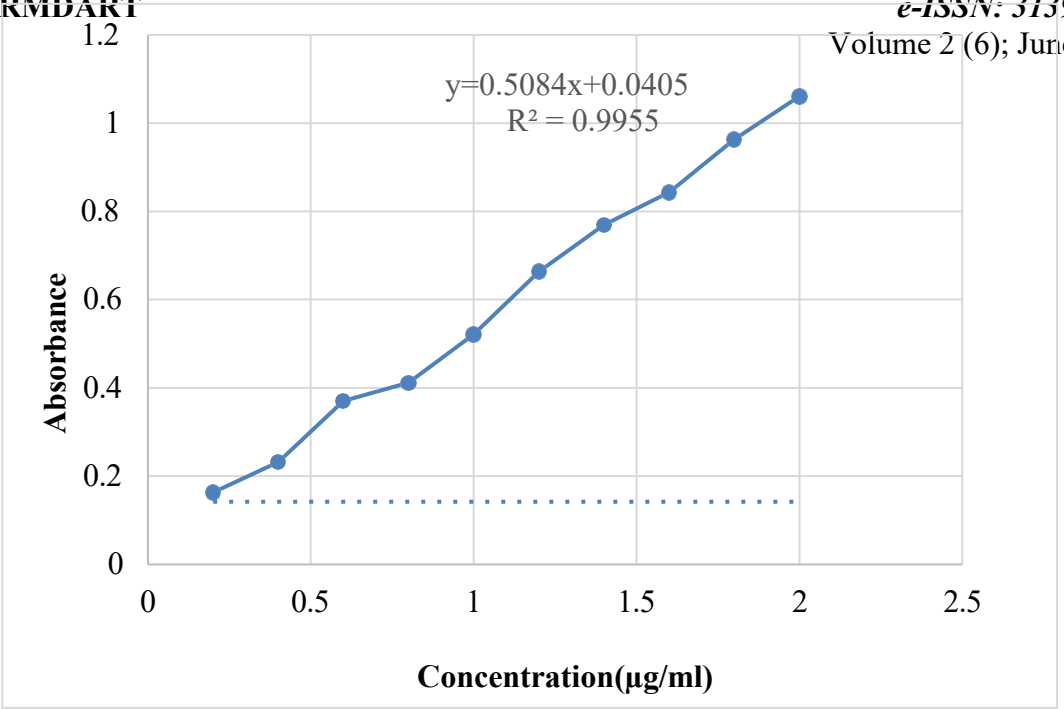

Fig6.2Graph-Standard Curve of Gallic acid

Estimation of Total Alkaloid Content

The standard absorbance values of atropine are measured at concentrations ranging from1to 5 µg/ml. At 1 µg/ml, the absorbance is 0.056, which increases progressively with concentration. The absorbance values are 0.163 at 2 µg/ml, 0.275 at 3 µg/ml, 0.384 at 4 µg/ml, and 0.498 at 5 µg/ml, indicating a linear relationship between concentration and absorbance (Graph 3 – Standard Curve of Atropine). The *Silybum marianum* extract shows absorbance values of 0.018, 0.023, and 0.025 in triplicate readings. Based on the calibration curve, the total alkaloid content of the extract is calculated as 70.86 µg atropine equivalent (AE)/ml. The mean value is 70.86 with a standard deviation of 3.26, and the result is expressed as 70.86 ± 3.26 (AE µg/ml).

S. No.	Concentration(µg/ml)	Absorbance
1.	1	0.056
2.	2	0.163
3.	3	0.275
4.	4	0.384
5.	5	0.498

Table6.7Standard Absorbance of Atropine

Sample	Absorbance (y)	Mean	Standard Deviation	Mean±SD (AEµg/ml)
S.M. 1	0.018	70.86	3.26	70.86 ± 3.26
S.M. 2	0.023			
S.M. 3	0.025			

Table6.8 Total Alkaloid Content of *Silybum marianum* extrac

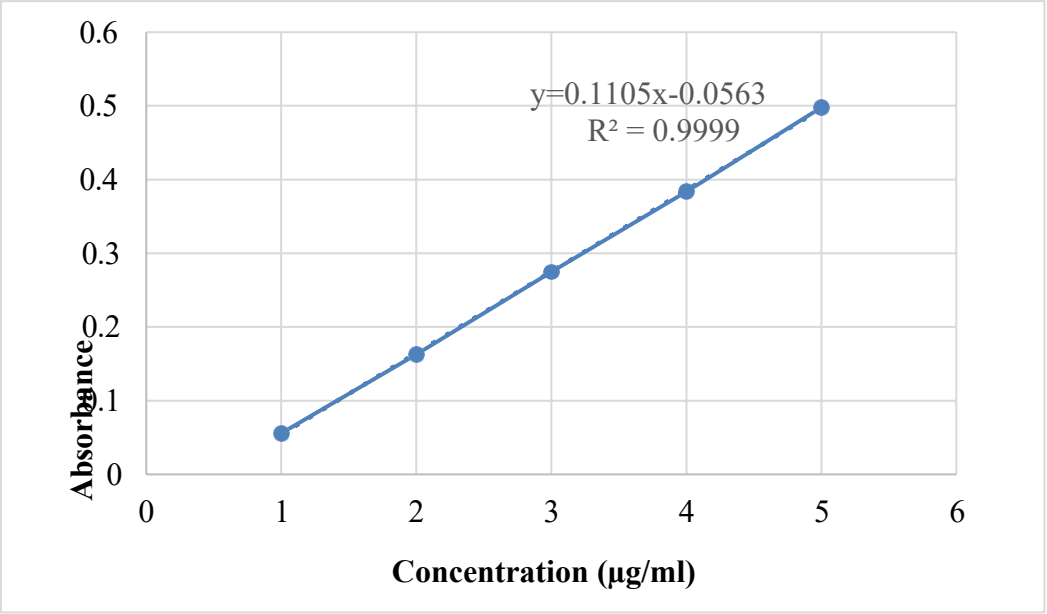

Fig6.3 Graph-Standard Curve of Atropine

Estimation of Total Tannin Content

The standard absorbance values of tannic acid are recorded at concentrations ranging from 0.2 to 2.0 µg/ml. at 0.2 µg/ml, the absorbance is 0.002, and it increases consistently with concentration. The absorbance values are 0.138 at 0.4 µg/ml, 0.241 at 0.6 µg/ml, 0.352 at 0.8µg/ml, 0.468 at 1.0 µg/ml, 0.577 at 1.2 µg/ml, 0.682 at 1.4 µg/ml, 0.764 at 1.6 µg/ml, 0.872at 1.8 µg/ml, and 0.969 at 2.0 µg/ml, showing a linear increase with concentration (Graph 4 – Standard Curve of Tannic Acid). The *Silybum marianum* extract shows absorbance values of 0.195, 0.198, and 0.201 in triplicate readings. Based on the calibration curve, the total tannin content of the extract is calculated as 52.01 µg tannic acid equivalent (TAE)/ml. The mean value is 52.01 with a standard deviation of 0.56, and the result is expressed as 52.01 ± 0.56 (TAE µg/ml).

S. No.	Standard Concentration (µg/ml)	Absorbance
1.	0.2	0.002
2.	0.4	0.138
3.	0.6	0.241
4.	0.8	0.352
5.	1	0.468
6.	1.2	0.577
7.	1.4	0.682
8.	1.6	0.764
9.	1.8	0.872
10.	2	0.969

Table6.9 Standard Absorbance of Tannic acid

Sample	Absorbance(y)	Mean	Standard Deviation	Mean±SD (TAE µg/ml)
S.M. 1	0.195	52.01	0.56	52.01 ± 0.56
S.M. 2	0.198			
S.M. 3	0.201			

Table6.10 TotalTanninContentof*Silybummarianum* extract

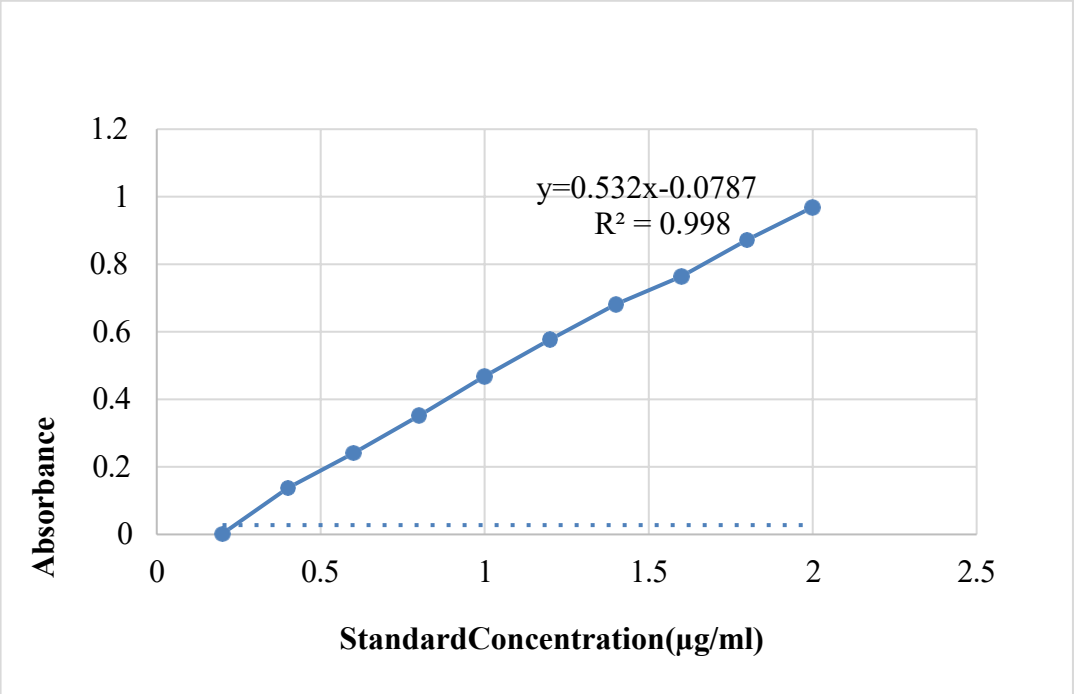

Fig6.4 Graph –Standard Curve of Tannic acid

Estimation of Total Saponin Content

The standard absorbance values of vanillin (5% w/v in glacial acetic acid) are recorded at concentrations ranging from 0.2 to 2.0 µg/ml. At 0.2 µg/ml, the absorbance is 0.163, and it increases progressively with concentration. The absorbance values are 0.232at0.4µg/ml, 0.370 at 0.6µg/ml, 0.412 at 0.8µg/ml,0.521at1.0µg/ml,0.663 at 1.2µg/ml,0.769 at1.4µg/ml, 0.843 at 1.6 µg/ml, 0.963 at 1.8 µg/ml, and 1.061 at 2.0 µg/ml, showing a linear increase with concentration (Graph 5 – Standard Curve of Vanillin). The *Silybum marianum* extract shows absorbance values of 0.274, 0.273, and 0.278 in triplicate readings. Based on the calibration curve, the total saponin content of the extract is calculated as 62.06 µg equivalent/ml. The mean value is 62.06 with a standard deviation of 0.52, and the result is expressed as 62.06 ± 0.52 µg/ml.

S. No.	Standard Concentration (µg/ml)	Absorbance
1.	0.2	0.163
2.	0.4	0.232
3.	0.6	0.37
4.	0.8	0.412
5.	1	0.521
6.	1.2	0.663
7.	1.4	0.769
8.	1.6	0.843
9.	1.8	0.963
10.	2	1.061

Table6.11 Standard Absorbance of Vanillin

Sample	Absorbance(y)	Mean	Standard Deviation	Mean±SD (VEµg/ml)
S.M. 1	0.274	62.06	0.52	62.06 ± 0.52
S.M. 2	0.273			
S.M. 3	0.278			

Table6.12 Total Saponin Content of *Silybum marianum* extract

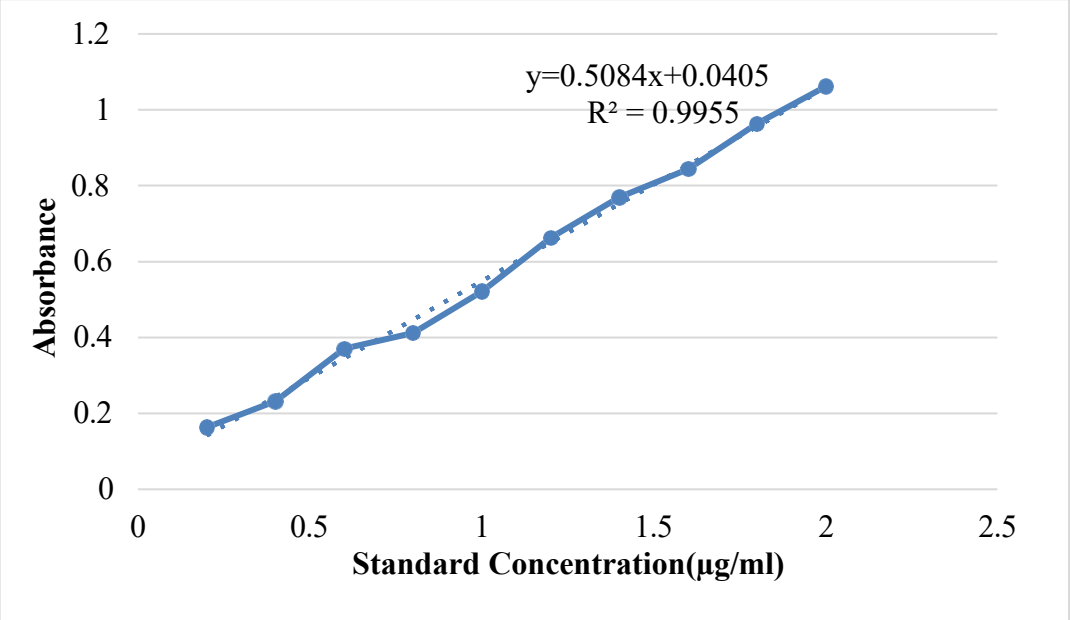

Fig6.5Graph –Standard Curveof Vanillin

Estimation of Total Ash Value

The total Ash value of *Silybum marianum* is determined using the incineration method at a temperature of 600 ℃. The analysis shows that the plant contains 6.4% total ash, which represents the inorganic residue remaining after complete combustion of the plant material.

Table6.13 Total Ash value

Plant Name	Estimation Method	Temperature	Total Ash Value
Silybummarianum	Incineration Method	600℃	6.4%

Physico-chemical properties of silymarin Physico-chemical properties of silymarin were assessed and following outcomes were observed:

Organoleptic properties of silymarin.

Appearance - Fine powder

Colour - Darkyellow

Odour -Odourless

Melting point It was determined using melting point apparatus and it was found to be 164 - 165˚C which is in agreement with the value reported by Yang et al. (2015) i.e., 164 - 174˚C.

Solubility

Solubility of silymarin invarious solvents was examined. The details are mentioned in Table 6.14.

Solvent	Solubility
Water	0.038± 0.888 mg/mL
PBS(pH 7.4)	0.206± 0.769 mg/mL
PBS(pH 6.8)	0.155± 0.444 mg/mL
Methanol	2.441± 0.982 mg/mL

Table6.14 Solubility of silymarin in different solvents

Partition coefficient

The partition coefficient of silymarin was found to be 1.91 which is close to the reported value i.e., 2.2 (Gazak et al., 2004). Silymarin is moderately lipophilic in nature with log P >1.

Spectral analysis of silymarin

UVanalysis

λmax of silymarin in methanol A silymarin solution with concentration 20 µg/mL was scanned using UV spectrophotometer (UV-1800, Shimadzu) from 200-400 nm andλmax of silymarin was found to be 288.2 nm (Figure 6.6). The reported λmax is 288 nm (Ahmad et al., 2023).

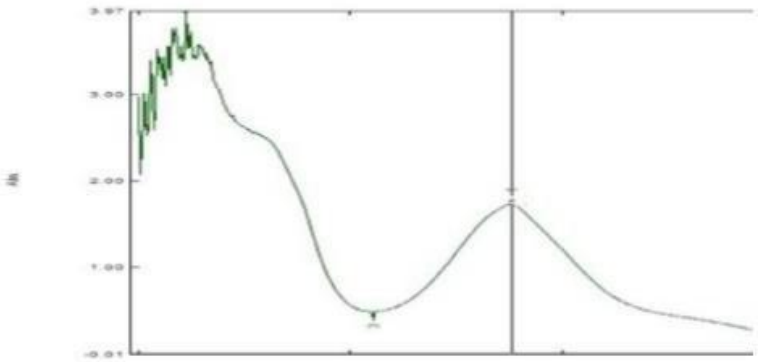

Fig6.6 UV spectrum of silymarin in methanol

Standard plot of silymarinin methanol

The absorbance values corresponding to each concentration is enlisted in Table 6.15. Standard plot

of silymarin in methanol was constructed by crafting concentration (µg/mL) at X axis and absorbance at Y axis (Figure 6.7)

Conc.(µg/mL)	Absorbance
2	0.167
4	0.277
6	0.456
8	0.578
10	0.677
12	0.833
14	0.938
16	1.084

Table6.15UVanalysis of silymarinin methanol

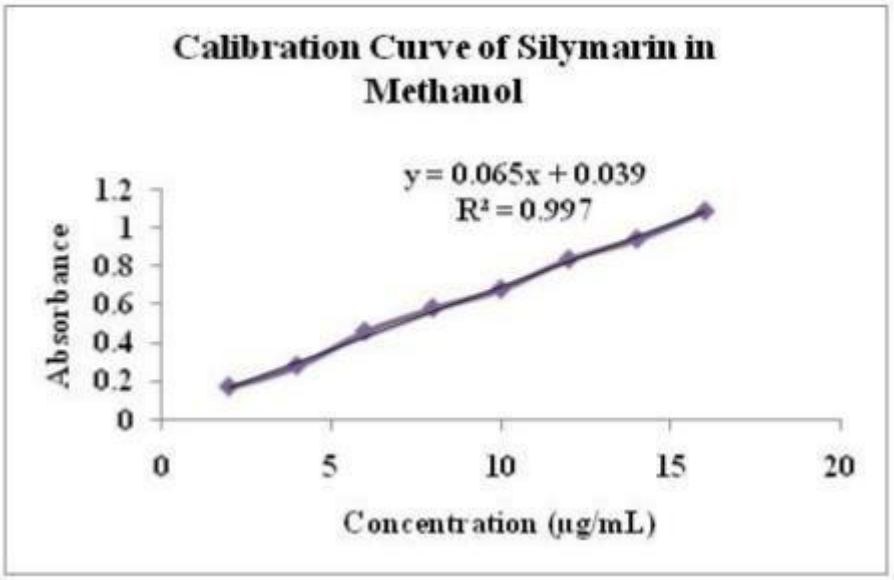

Fig6.7 Calibrationcurveofsilymarinin methanol

Infrared analysis

The FTIR spectrum of silymarin is illustrated in Figure 6.8. The characteristic peaks present in the spectrum are mentioned in Table 6.16. All the peaks of the principle functional groups present in the silymarin structure are indicated in the spectrum confirming its identification.

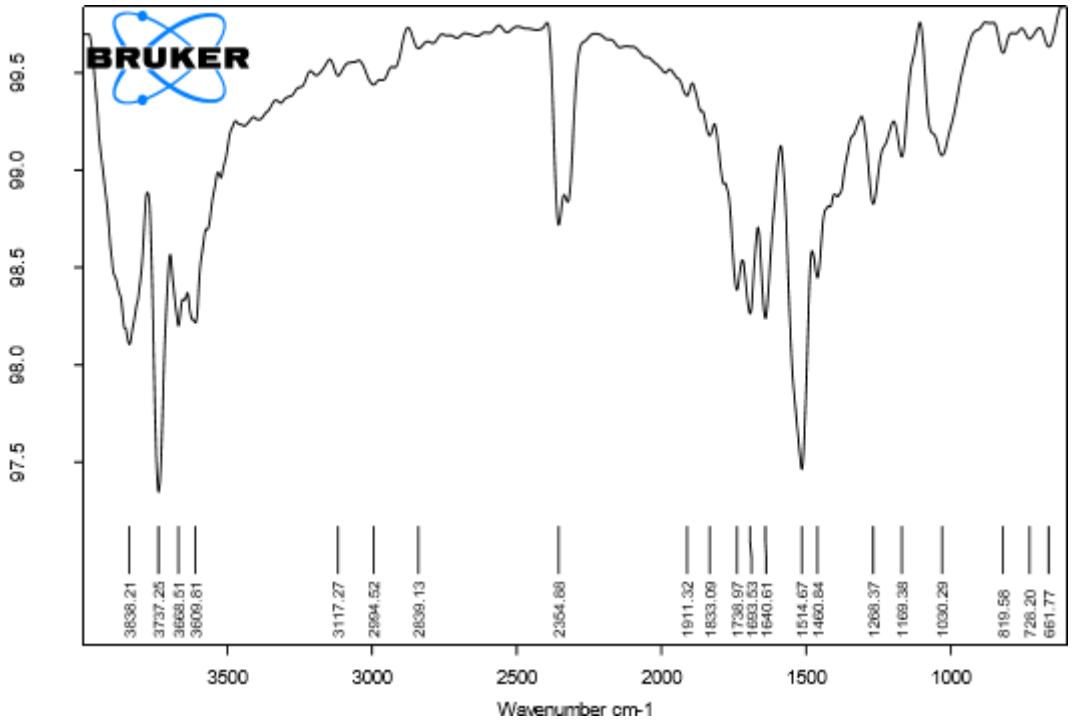

Fig6.8 FTIR spectrum of silymarin

Groups	AbsorbanceRange (cm-1)	ObtainedAbsorbance (cm-1)
AlcoholOHstretch (free)	3700 -3584	3609.81
C=Caromatic	1625 – 1440	1460.84
C-O-Cstretch	1250 -1050	1191.32
C=Oketone	1750-1705/1700-1665	1738.97
-OCH3	2860-2800	2839.13
AromaticCHstretch/CHbend	900 – 680/ 3100 -3000	819.58/3117.27
AlkanesC-Hstretch	2940 – 2850	2994.52

Table6.16 Characteristic peaks represent in the FTIR spectrum of silymarin

Particle size distribution

The particle size of the prepared Silymarin Extract Phytosomes (SEP) was carried out using dynamic light scattering technique and showed in figure 6.9. The mean particle size of SEP was distributed in a narrow range found to be 233.4±20.0 nm, and polydispersity index was 0.642±0.03.

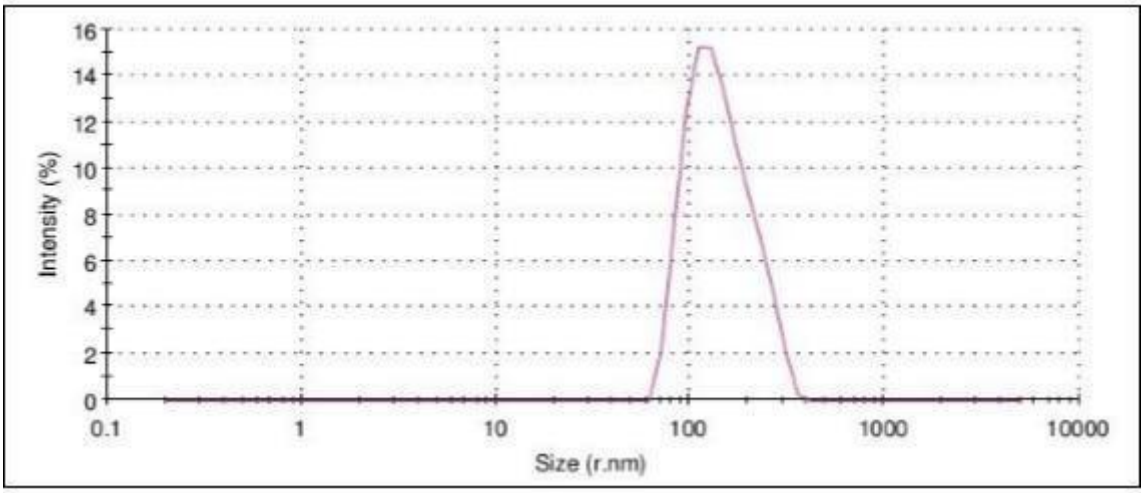

Fig.6.9 Particle size analysis of SEP

S. No.	Material	Avg.Size (r nm)	%Intensity	Width (r.nm):	PDI
1	SEP	233.4±20.0	100	55.95	0.642±0.03

Table 6.17 Particle size analysis of SEP.

Differential scanning calorimetry (DSC)

The Differential scanning calorimetry (DSC) of SEP are shown in figure 6.10. The DSC thermograms of SEP showed figure 24gives two endothermic peaks at 206.40°Cand 244.54°C. Therefore, from figure 6.10 it was revealed that the shift of endothermic peak at the difference of around 25-30°C suggest possible interaction of Silymarin with Phospholipids and can account for enhanced entrapment.

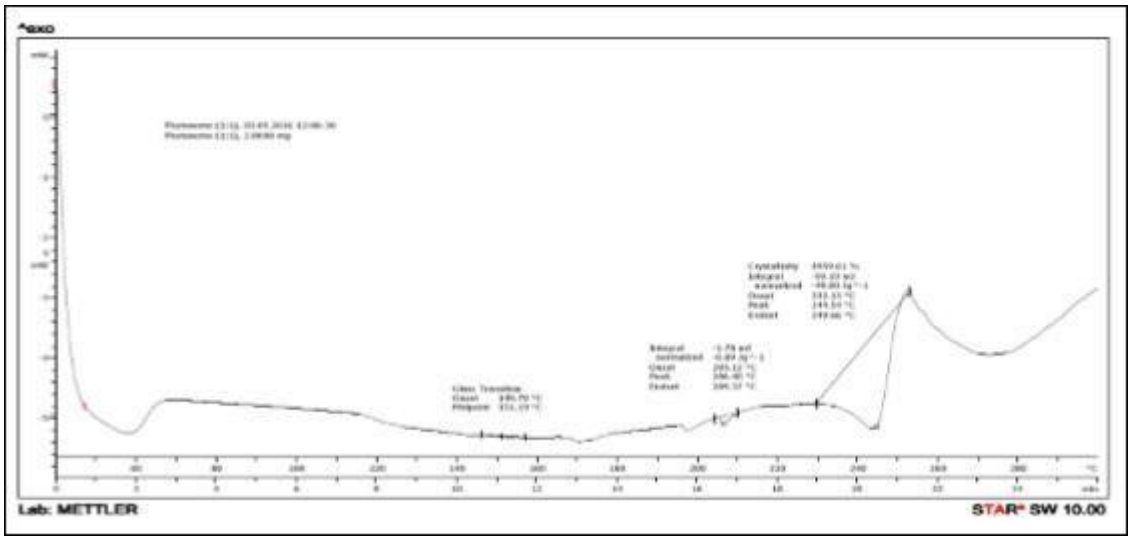

Fig.6.10 Differentialscanningcalorimetryof SEP

Percentage drug release

The results of in vitro drug release studies are shown in figure 6.11 and table 6.18. The 12-h dissolution in the phosphate buffer (pH 6.8) revealed that, the pure SE (Silymarin extract) showed the slowest rate of dissolution, i.e., at the end of the dissolution period only about 44% w/w of SE was dissolved. The dissolution rate of the physical mixture was found not to be significantly different (49%w/w dissolved in12h) compared to the pure SE. The prepared SEP revealed a significantly faster release of SE at the end of dissolution period. At the end of 12 h, over 89% w/w SE was observed to be released from the SEP. The dissolution rate is largely influenced by the crystal morphology and the wettability of the solids, 44 and the improved dissolution rate of SE from the SEP may be explained by the improved solubility, and the amorphous form in the prepared complex. The relatively higher amorphous state of the phytosome and the increased aqueous solubility may have a positive impact on the cumulative release of the drug.

Time (inHr.)	%Drug Release	
	SE	SEP
1	22.0811	35.1784
2	29.4324	40.3212
3	34.3243	46.3242
4	36.4054	57.2367
5	38.6487	63.1324
6	39.1892	67.4532
7	41.3478	70.1276
8	42.4653	73.3498
9	42.7236	75.6532
10	43.3489	80.5609
11	44.1256	85.5432
12	44.7896	89.7865

Table 6.18 *Invitro* dissolution study of SE and SEP

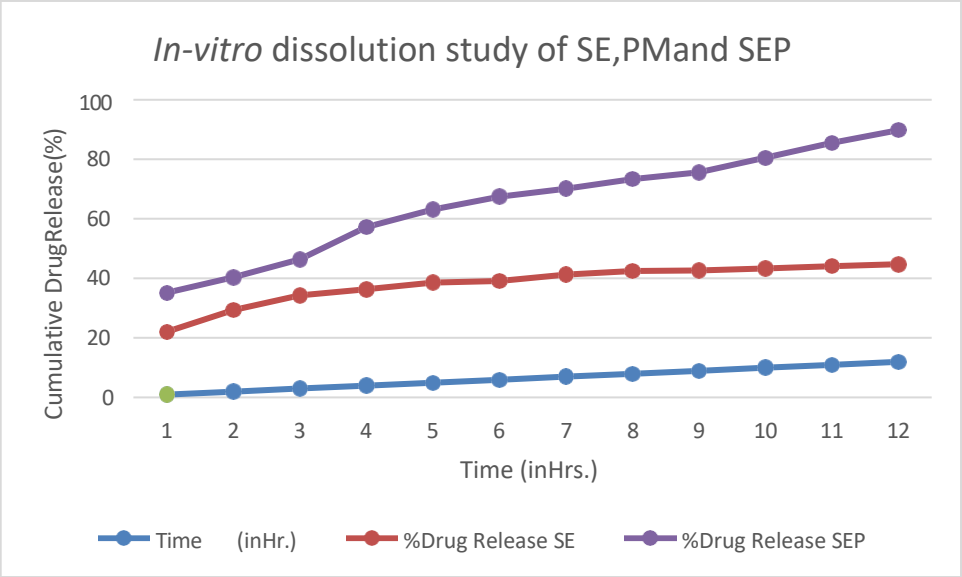

Fig.6.11 Invitro dissolution study of SE and SEP

Assessment of antioxidant activity

DPPH Assay

DPPH radical scavenging activity of extract was showed in Figure 6.12 and Table 6.19. DPPH assay was determining the antiradical power of an antioxidant by measuring the absorbance of DPPH. The % scavenging effect of DPPH radical increase as the concentration of SEP, SE and vitamin C is increase in the order of vitamin C >SEP > SE, which were 95.56%, 92.74% and 74.43%, at the concentration of 1000 µg/mL, respectively.

Table6.19DPPH radicals cavenging activity SCE, CP and Vit-C

S.No.	Concentration(µg/ml)	DPPH(%Inhibition)		
		SE	SEP	Vit. C
1	100	17.21±0.16	19.70±0.23	23.92±0.13
2	200	31.23±0.31	48.09±0.43	54.68±0.14
3	400	51.57±0.17	63.18±0.21	68.04±0.17
4	600	62.32±0.02	72.18±0.03	78.08±0.15
5	800	70.08±0.22	82.62±0.32	85.34±0.46
6	1000	74.43±0.14	92.74±0.42	95.56±0.26

*All values are mean ±SD (n=3)

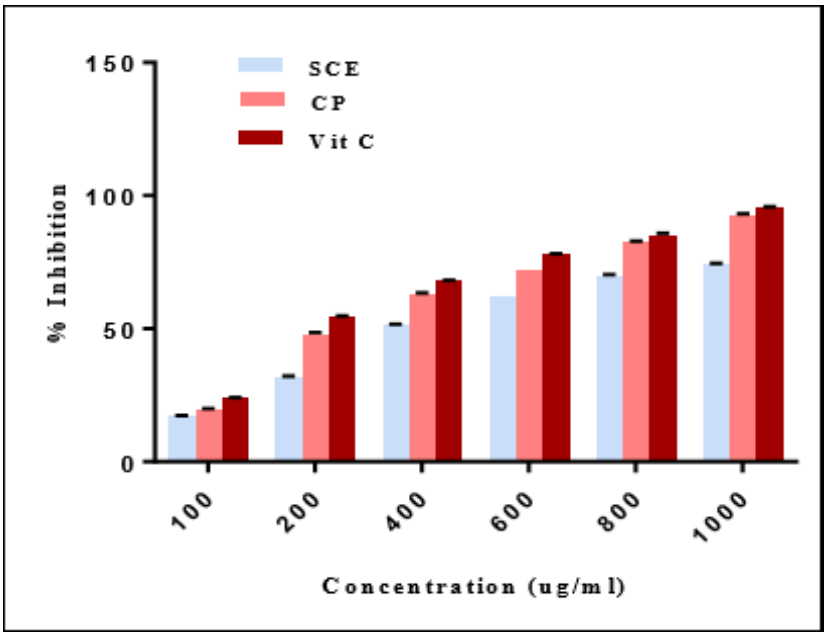

Fig.6.12DPPH radical scavenging activity of SE and SEP

CONCLUSION:

The present study was carried out with the aim of developing a novel herbal drug delivery system of *Silybum marianum* (silymarin) in the form of phytosomes, in order to improve its physicochemical properties and enhance its therapeutic potential for liver disorders,especially liver cirrhosis.

The phytochemical investigation of the ethanolic extract confirmed the presence of several important bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, saponins, terpenoids, and glycosides. These constituents are widely recognized for the antioxidant and hepato protective properties and play a vital role in protecting liver cells from oxidative stress and toxic injury. Quantitative analysis further showed that flavonoids were present in higher amounts, supporting the traditional use of silymarin as a liver-protective agent.

Physicochemical evaluation of silymarin revealed poor aqueous solubility and moderate lipophilicity, which are major limitations affecting its oral bioavailability. This justified the need for an advanced delivery approach. Spectral studies, including UV and FTIR analysis, confirmed the identity and structural integrity of silymarin, ensuring its suitability for formulation development.

The successfully developed Silymarin Extract Phytosomes (SEP) showed nanosized particle distribution with acceptable uniformity. The interaction between silymarin and phospholipids, as confirmed by DSC analysis, indicated the formation of a stable phytosomal complex. This interaction plays an important role in improving drug solubility, stability, and absorption.

Invitro dissolution studies clearly demonstrated that the phytosomal formulation significantly enhanced drug release when compared with the plain silymarin extract. Nearly complete drug release

was achieved from the phytosomes, whereas the conventional extract showed limited dissolution. This improvement can be attributed to better wettability, reduced crystallinity, and partial conversion of the drug into an amorphous form.

The antioxidant activity evaluated by the DPPH assay further supported the effectiveness of the developed formulation. The phytosomal system exhibited markedly higher free radical scavenging activity than the pure extract and showed activity comparable to that of the standard antioxidant. Since oxidative stress is one of the major contributing factors in the development and progression of liver cirrhosis, the enhanced antioxidant potential of the phytosomal formulation is of significant therapeutic importance.

Future Prospects

Liver cirrhosis is a chronic and progressive disease characterized by continuous liver injury, inflammation, oxidative stress, and fibrotic tissue formation. Current treatment options mainly focus on slowing disease progression rather than reversing liver damage. In this context, herbal-based novel drug delivery systems offer a hopeful direction for long-term liver management.

The encouraging results obtained in this study suggest that silymarin phytosomes may have strong potential in the treatment of liver cirrhosis. In future studies, the formulation can be evaluated in experimental models of cirrhosis to assess its protective effect on liver architecture, enzyme levels, and fibrotic markers. Improved bioavailability may allow better intracellular delivery of silymarin to hepatocytes, thereby enhancing its anti-fibrotic and regenerative actions.

Further investigations may also explore liver-targeted phytosomal systems to achieve site-specific delivery, reducing systemic exposure and improving therapeutic efficiency. Combination therapy using phytosomes loaded with multiple hepatoprotective phytoconstituents may provide synergistic benefits in controlling inflammation, fibrosis, and oxidative damage associated with cirrhosis.

With detailed in vivo studies, long-term safety evaluation, and clinical validation, silymarin phytosomes may emerge as a safe, effective, and patient-friendly herbal therapeutic option for the supportive management and progression control of liver cirrhosis.

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